Biohistorical materials and contemporary privacy concerns - The forensic case of King Albert I

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Abstract

The rapid advancement of technology in genomic analysis increasingly allows researchers to study human biohistorical materials. Nevertheless, little attention has been paid to the privacy of the donor's living relatives and the negative impact they might experience from the (public) availability of genetic results, even in cases of scientific, forensic or historical relevance. This issue has become clear during a cold case investigation of a relic attributed to Belgian King and World War I-hero Albert I who died, according to the official version, in a solo climbing accident in 1934. Authentication of the relic with blood stains assigned to the King and collected on the place where his body was discovered is recognised as one of the final opportunities to test the plausibility of various conspiracy theories on the King's demise. While the historical value and current technological developments allow the genomic analysis of this relic, publication of genetic data would immediately lead to privacy concerns for living descendants and relatives of the King, including the Belgian and British royal families, even after more than 80 years. Therefore, the authentication study of the relic of King Albert I has been a difficult exercise towards balancing public research interests and privacy interests. The identification of the relic was realised by using a strict genetic genealogical approach including Y-chromosome and mitochondrial genome comparison with living relatives, thereby limiting the analysis to genomic regions relevant for identification. The genetic results combined with all available historical elements concerning the relic, provide strong evidence that King Albert I was indeed the donor of the blood stains, which is in line with the official climbing accident hypothesis and contradicts widespread 'mise-en-scène' scenarios. Since publication of the haploid data of the blood stains has the potential to violate the privacy of living relatives, we opted for external and independent reviewing of (the quality of) our data and statistical interpretation by external forensic experts in haploid markers to guarantee the objectivity and scientific accuracy of the identification data analysis as well as the privacy of living descendants and relatives. Although the cold case investigation provided relevant insights into the circumstances surrounding the death of King Albert I, it also revealed the insufficient ethical guidance for current genomic studies of biohistorical material.

Introduction

The rapid advancement of technology in genomic analysis increasingly allows researchers to study human biohistorical materials [1, 2]. Genomic analyses of historical remains have already proved to be highly valuable to archaeological research (e.g. the identification of the remains of the English King Richard III [3]), historical studies (e.g. the fate of Louis XVII during the French revolution [4]), scientific investigation (e.g. the first sequence of an Aboriginal genome using 90-year-old hair [5]) and forensic research (e.g. the identification of the remains of British and Australian soldiers who died during World War I [6]). Despite these benefits, genetic analyses can violate the privacy of the historical person of concern, without any kind of informed consent. For deceased persons, a long time interval after the death of the person ('death + time'-approach) is often proposed to override the absence of his or her consent, but at present, the length of this timeframe (10-100 years after dead) is still subject to debate [7-9].

In these debates, little attention has been paid to the privacy of living relatives and the harm they might experience from the (public) availability of genetic results [7, 10]. For example, publication of male Y chromosome can reveal (incorrect) relatedness with a living male person, or otherwise, publication of a detected mutation that causes a medical anomaly reveals a higher chance of the descendants to carry the same mutation. Hence, some ethical guidelines have been suggested to ensure the scientific relevance of biohistorical analyses, warning against sensationalism, purely commercial purposes and studies with research questions that can be properly answered by the analysis on living relatives [10, 11]. Nevertheless, it is still not clear how to approach the analysis and publication of genetic data in such cases which have a high scientific, historical or forensic relevance but have at the same time the potential to harm living relatives. This issue has become clear in the case of a relic related to the Belgian King Albert I which we present here.

In this study, genetic material related to the death of King Albert I of Belgium (1875-1934) has been analysed. Albert I was King between 1909 and 1934 and had a major role during World War I by refusing German troops to move through Belgium to France (Figure 1a) [12]. He received an enormous international esteem and recognition because of his military and diplomatic achievements [13], but also for his post-war politics supporting peace, democracy

and science [14]. King Albert I died unexpectedly, which caused a worldwide mourning, demanding for a detailed description of the circumstances of his death. According to the official version he died from a climbing accident while he was exercising alone on the rocks [Official juridical file, Archive of the Royal Palace, Brussels, Belgium]. Witnesses testify to have found his body at the foot of a rock during the night of 17 and 18 February 1934 near the village Marche-les-Dames [15, 16], where since then a highly popular memorial was established (Figure 1b). Immediately after his death, however, various conspiracy theories casted doubts on the official version, suggesting that the King was murdered (or even committed suicide) somewhere else and that his body has never been at Marche-les-Dames, or that it was deposited over there [17, 18]. Several of those hypotheses with criminal motives were investigated by the juridical authorities but the doubts have been increased ever since, today still being the subject to popular novels, books and documentaries [19-22]. The circumstances of the demise – the absence of eye-witnesses, a juridical investigation that is far from flawless compared to current standards and many irregularities in the followed procedure after the discovery of the body – surely paved the way for rising conspiracy theories.

In several private collections and museums (e.g. Royal Museum of the Armed Forces and of Military History, Brussels), relics are still present including leaves with blood stains assigned to King Albert and collected by local residents on the place where his dead body was discovered. DNA analysis on these blood stains is widely considered by historians and adherents of conspiracy theories as one of the few remaining possibilities to get more clarity on the circumstances of this case after more than 80 years [19]. Although the importance of blood patterns in a forensic case was already recognized in the 1930s [23], no technical report, photo or witness testimony described these traces properly during the juridical investigations [Official juridical case files, Archive of the Royal Palace, Brussels, Belgium]. This lack in the investigations is one of the main reasons why adherents of conspiracy theories declare that the finding of the body must be a complete 'mise-en-scène' [19]. These theories stated that the body of the supposedly murdered King had never been at Marche-les-Dames or - according to others - that it was translocated from Brussels, seven to nine hours after his decease and after the onset of *rigor mortis* [19,21,24]. Therefore, they stated that there were no blood stains at the foot of the rock in Marche-les-Dames or that they had a non-royal origin [19, 21, 24]. On the other hand, historians who support the climbing accident hypothesis pointed to the fact that many

traces like the blood stains on the place where the body was discovered were already disappeared before the start of the investigations by the authorities, so that there was no possibility to analyse them properly. Newspaper reports from 19 February 1934 stated indeed that local residents collected leaves with blood stains already during the night of the discovery of the body [e.g. Dutch newspaper 'Algemeen Handelsblad', 19th of February 1934, p1]. Nevertheless, only a positive identification of the blood stains is a breakthrough, though, giving support to the official version that the King died in Marche-les-Dames. A negative identification on the other hand does not necessarily reject the official version as it is expected that many false relics have been sold by local residents as souvenirs [19].

Next to the fact that the historical and forensic value of these relics with blood stains in this cold case is undeniable, an extensive genomic identification is technologically feasible and the performance of the analysis and publication of data is completely legal given the long time period since his dead (more than 80 years). Nevertheless, the study of such remains and the publication of a positively identified DNA sample would still immediately create various privacy concerns for living descendants and relatives of the King. First of all, the genetic data from the historical sample can be used to determine the paternity of and relatedness with different members of the royal family. Various claims and widely spread gossip of putative paternity can be addressed with this sample. The most notorious example is a current and highly mediatized court case where Delphine Boel claims to be the daughter of Albert II, the previous King of Belgium and living grandson of Albert I, and asked the court to get a DNA sample from Albert II or from his official descendants [25]. DNA data from blood samples assigned to Albert I can indeed be used in this famous case using genome-wide markers [26], however, it is highly unethical to use them without any informed consent of the living persons involved. Moreover, since biological relatedness is the only prerequisite to qualify as a royal heir and to be a member of the royal family, determining (fallacies in) the genetic royal descent can be highly harmful to the royal family. As for other studied important families [27], the possibility exists also to commercialise the Saxe-Coburg Y chromosome profile, which includes not only the Belgian royal family, but also the British (the House of Windsor) and the former Bulgarian and Portuguese royal families which are closely related to the Belgian. Next to relatedness issues, whole genome analysis of the relic can also detect inheritable conditions, susceptibilities to diseases and physical traits of Albert I. Public knowledge on

158 these traits could negatively affect or embarrass descendants [10]. Moreover, if (susceptibility
159 to) infertility would be discovered, the position of the present King – a legal great-grandson of
160 Albert I – would be threatened [7]. Also the emotional aspects of the descendants and the public
161 should be taken into account: public knowledge on predispositions to disease or characteristic
162 can harm the current King's heroic image[28-30].
163
164 In 2013, a journalist acquired on a public auction a sample of leaves with blood stains assigned
165 to the scene of the King's death in Marche-les-Dames in 1934. We aimed in this study to
166 genetically identify the royal blood sample in a highly qualitative scientific procedure and
167 report the analysis and results scientifically correct, but taking into account the privacy of the
168 descendants. The authentication of the royal relic was realised by means of a protein analysis
169 and a genetic genealogical approach using the full mitochondrial genome and (family-specific)
170 Y chromosomal variation.
171
172 **Materials & Methods**
173
174 ***Description of the sample.*** The relic with the blood sample used for genetic analysis was
175 acquired on a public auction in 2013. It consisted of two sheets with leaves and a soil sample
176 behind a broken glass framed in golden paper (120 mm x 90 mm) (Figure 3). The first sheet
177 contained three leaves from the common beech (*Fagus sylvatica*) and the second a soil
178 sample, three leaves of ivy (*Hedera helix*) and a small beech leaf. Beech and ivy grew on the
179 exact place where the King's body was found, as shown by pictures of the juridical report
180 [Official juridical case files, Archive of the Royal Palace, Brussels, Belgium], and are still
181 commonly present on the location today (pers.obs. July 2015). An authentication certificate
182 was hidden between the two sheets and was signed with three names (Figure 4). One of the
183 names had been identified as Mrs. Marthe de Thier, maiden name Facq (1880-1957), a
184 noblewoman from Namur. At the time of the King's demise she lived about 7 km off the place
185 of the accident in Marche-les-Dames, and was a close neighbour of the technical expert for
186 the juridical investigation on the death of Albert I, Albert Fisette [Official juridical case files,
187 Archive of the Royal Palace, Brussels, Belgium]. Together with her husband Henri de Thier
188 (1869-1941), who was a retired colonel of the Belgian army, she possessed a big network of
189 influential persons and had a high esteem in the region of Namur [31, 32]. The second name was

written in an illegible handwriting but the small letter 'd' as the first letter points most likely to a person of nobility as well. The last name, Goossens R, has not yet been identified since civil records in Belgium are not available until 100 years old.

Ethics statement. A genetic analysis of the sample was positively advised by the interdisciplinary bio-ethical committee of KU Leuven – University of Leuven (Commissie voor medische ethiek; date 30th of September 2014; chairman: Prof. Martin Hiele).

Protein analysis. Potential blood traces were sampled on the relic and further processed for proteome analysis using mass-spectrometry as described in Van Steendam *et al.* [33].

Contamination precautions. Genetic analyses were performed in the ancient DNA facilities of the Laboratory of Forensic Genetics and Molecular Archaeology in Leuven. Standard contamination precautions as described in literature were employed for the whole molecular analysis [34-36], amongst which: pre- and post-PCR procedures carried out in physically separated laboratories, restricted and controlled access to the pre-PCR laboratory, mock-extraction controls in the DNA extractions and amplification reactions, and independent replication of data. More details about contamination avoidance procedures in the ancient DNA facilities in Leuven are reported in Ottoni *et al.* [37].

DNA extraction and quantification. Blood samples of the relic and buccal swab DNA samples of two reference persons were collected for DNA extraction by using the Maxwell 16 System (Promega, Madison, WI, USA). All DNA extractions were followed by real-time PCR quantification (Quantifiler Human DNA kit, Applied Biosystems, Foster City, CA, USA).

Mitochondrial control region analysis. Analysis of the first and the second hypervariable segments (HVS-I and HVS-II) of the mitochondrial DNA (mtDNA) control region of the blood sample attributed to King Albert I was accomplished by amplifying and direct sequencing, respectively, five and two overlapping fragments (ranging from 109–166 bp in size), in order to read, respectively, 357 bp, from nucleotide position (np) 16009–16365, and 217 bp, from np 49–265. List of primers used and details about the amplification, sequencing and

minisequencing reactions are reported in Ottoni *et al.* [37]. Sequencing was carried out using the Big-Dye Terminator V3.1 cycle sequencing kit (Applied Biosystems) and by capillary electrophoresis on an ABI Prism 3130XL Genetic Analyser (Applied Biosystems). The HVS-I and HVS-II sequences were compared with earlier published ones of anonymous maternal relatives of King Albert I [38].

Mitochondrial genome analysis. The whole-genome sequencing of the mtDNA sequences of the blood sample and of the buccal swab sample from Anna Maria, Freifrau von Haxthausen, who is separated eight meioses from King Albert I in direct maternal line (Figure 5), was carried out following the target capture method of Bekaert *et al.* [39], which is based on the method of Maricic *et al.* [40]. The only adjustment was the use of the NEBNext Ultra DNA library Prep Kit for Illumina in the library preparation (New England BioLabs, Ipswich, Massachusetts, USA). The libraries were sequenced on an Illumina Miseq platform at the sequencing facility 'Genomics Core' of the KU Leuven and UZ Leuven, Belgium. Reads for both reference and blood sample were mapped to the revised Cambridge reference mitochondrial sequence (NC012920.1) using Geneious software v.6.1.8.

Y-chromosome haplotype analysis. A set of 42 Y-STR loci were genotyped for the blood samples as well as afterwards on a buccal swab sample of King Simeon of Saxe-Coburg and Gotha, who is separated eight meioses from King Albert I in direct paternal lineage (Figure 5). They were genotyped as described in previous studies [41, 42] but instead of using PowerPlex® Y (Promega Corporation), primers for the Y-STRs of the recently developed PowerPlex® Y23 System (Promega Corporation) were included in three overlapping multiplex reactions together with Y-STRs used in previous studies [43, 44]. The list of all 42 Y-STR loci is given in supplementary materials, as well as the list of the 29 Y-STRs for which we could independently replicate the allele values for the blood sample. To avoid any contamination, the haplotype analysis was separately done for the blood sample in August 2014 and for the reference sample in September 2015. None of the researchers involved in the DNA extraction had also an identical profile; the person who did the Y-STR analysis is a female. We compared the 29 Y-STR haplotypes of the blood donor and the reference individual with each other. Based on the calculated mean mutation rate for the 29 genotyped Y-STRs using the individual mutation rates measured by Ballantyne *et al.* [45], namely 5.48 x 10^{-3} mutations per generation, the

number of meioses in direct patriline (95% credibility interval) between both individuals was calculated using the formulae of Walsh [46].

Y-chromosome SNP analysis. The haplotypes were submitted to Whit Athey's Haplogroup Predictor [47, 48] to obtain probabilities for the inferred haplogroups. The samples of the relic and the reference person were assigned to specific Y-SNPs assays to confirm the inferred haplogroup and to assign the sub-haplogroup according to the finest phylogenetic level of the Y chromosomal tree published in van Oven *et al.* [49] (www.phylotree.org/Y). The Y-SNP multiplex systems were genotyped using SNaPshot mini-sequencing assays (Thermo Fisher Scientific) and ABI Prism 3130XL Genetic Analyser (Applied Biosystems), according to our previous publications [42, 50].

Results

Protein analysis. Using mass-spectrometry based proteome analysis as described by Van Steendam *et al.* [33], the presence of blood, and specifically human blood, on the tested samples taken from the relic could be confirmed by the presence of human haemoglobin subunit alpha (protein score 210, 4 peptides) and other human proteins (e.g. Serum albumin). In addition to human blood derived proteins, also human keratins were identified.

Female-line relatives and mtDNA analysis. We carried out mtDNA analyses in two stages. In the first stage, the first and the second hypervariable segments (HVS-I and HVS-II) of the mitochondrial DNA (mtDNA) control region of the blood sample attributed to King Albert I were sequenced in duplicate using Sanger sequencing. No sequence differences were observed between either duplicated samples. Sanger sequencing of cloned PCR products was also performed and no sequence differences were observed. We found a perfect HVS-I and HVS-II match between the blood sample and two anonymous maternal relatives of King Albert I published in a study by Weichhold *et al.* [38].

To determine the full mtDNA similarity, we carried out complete mitochondrial genome sequencing of the blood sample and of the buccal swab sample from Anna Maria, Freifrau von Haxthausen. A perfect whole mitochondrial sequence match was observed between the blood

sample and the maternal relative. The HVS-I and HVS-II fragments of both samples completely match the ones reported in Weichhold *et al.* [38]. More details about the mitochondrial genome analysis is given in supplementary materials.

We investigated the probability that the mtDNA match between the blood sample and the maternal relative could have occurred by chance. Four matches with the observed sequence were found in a database of 13,829 West Eurasian mtDNA complete control region sequences (http://empop.org/, Version 3 - Release 11) [51]. The HVS-I/II haplotype resulted in 4 matches in 15,832 West Eurasians. These results provide evidence that the donor of the blood stains and the reference donor, Anne Maria, Freifrau von Haxthausen, both belong to a rare mitochondrial haplotype.

Male-line relatives and Y chromosome analysis. We carried out a Y chromosome analysis on the blood sample and afterwards on a buccal swab sample of King Simeon of Saxe-Coburg and Gotha. Alleles of 29 out of 42 tested Y-STR loci were independently replicated for the blood sample (list of the 29 Y-STRs in supplementary materials). No alleles could be observed in the analysis for the 13 remaining Y-STRs, most likely due to the degraded DNA as these markers targeted those fragments with the longest lengths in our multiplexes. The 29 Y-STR haplotype matched completely with the one of King Simeon, for which all 42 Y-STRs were genotyped afterwards.

The samples of the relic and the reference person which were genotyped with Y-SNP assays, were assigned as well to the same haplogroup and sub-haplogroup according to the finest phylogenetic level of the Y chromosomal tree published in van Oven *et al.* [49] (www.phylotree.org/Y) which is currently the reference for forensic identification [52].

Using the formulae of Walsh [46] and the calculated mean mutation rate for the 29 genotyped Y-STRs using the individual mutation rates measured by Ballantyne *et al.* [45], namely 5.48 x 10^{-3} mutations per generation, there is 95% probability that the number of meioses in direct paternal line between the two individuals with identical 29 Y-STR haplotypes is between 0 and 20. Finally, we investigated the probability that the Y-STR match between the blood stain and the paternal relative could have occurred by chance. There were 19 matches with nine Y-STR haplotypes in the Yfiler database of YHRD (www.yhrd.org; YHRD release 51) [55] with 109,137 worldwide haplotypes (alleles for nine Y-STRs out of the 17 Y-STRs in the Yfiler were independently replicated for the blood sample). No matches with 14 Y-STR haplotypes in the

PowerPlex Y23 database of YHRD (www.yhrd.org; YHRD release 51) [55] with 26,869 worldwide haplotypes were observed (alleles for 14 Y-STRs out of the 23 Y-STRs in the PowerPlex Y23 were independently replicated for the blood sample). Also no matches with 14 Y-STR haplotypes in the Yfiler Plus database of YHRD (www.yhrd.org; YHRD release 51) [55] with 8,184 worldwide haplotypes were reported (alleles for 14 Y-STRs out of the 27 Y-STRs in the Yfiler Plus were independently replicated for the blood sample). No matches with the 29 Y-STR haplotypes were found in a database of 1,453 Y chromosomes from previous studies in Belgium and the Netherlands [50, 53, 54]. These results together with the assignment to the same subhaplogroup according to the finest phylogenetic level of the van Oven *et al.* [49] Y chromosomal tree, provide evidence that the donor of the blood stains and the reference donor, King Simeon, are indeed closely patrilineally related to each other (less than 20 meioses).

External revision of the data and statistical analysis

Since publication of the haploid data of the blood stains and of the reference donors has the potential to impact the privacy of living relatives, our data and statistical interpretation were reviewed independently by forensic experts in haploid markers – no co-authors of this study and working in (a) different institute(s) than any (co-)author of this study – to guarantee the objectivity of the identification analysis. The mitochondrial data were confidentially transferred to Prof Walther Parson, Institute of Legal Medicine, Innsbruck Medical University (Austria), curator of the EMPOP database (http://empop.org/). On 13/4/2016, Prof Parson confirmed the quality and correctness of the statistical interpretation of the mitochondrial genome data in this study (http://empop.org/, Version 3 - Release 11), as reported in this manuscript. The Y-chromosomal data were confidentially transferred to Prof Lutz Roewer, Institut für Rechtsmedizin, Charité Universitätsmedizin Berlin (Germany), curator of the YHRD database (www.yhrd.org). On 14/4/2016, Prof Roewer confirmed the quality and correctness of the statistical interpretation of the Y-chromosomal data (based on YHRD release 51), as reported in this manuscript. Both external reviewers declared to have made their revision of the data and statistical analysis completely independent, without any conflict of interest, and to have deleted all the transferred data after their analysis and revision. The signed statements of both external experts are attached as supplementary material.

Discussion

The authentication of the royal relic attributed to the Belgian King and World War I-hero Albert I was verified using firstly a protein analysis to prove that it was human blood and secondly a genetic genealogical approach. This second approach compared the full mitochondrial genome of the blood stains with that of a living maternal relative of Albert I, next to comparing (family-specific) Y chromosome variation with that of a living paternal relative of the King. Next to the molecular study, a historical and archival study has been done, focusing on the background of the collectors of the sample and the possibility of leaves with blood stains being collected, consulting the juridical case files on the death of Albert I, and public and private archives. The molecular and historical elements provide – separately and combined – strong evidence for the authenticity of the relic.

i) Historical and forensic value

The finding of authentic royal blood on the place debunks conspiracy theories that the King's body has not been at the location in Marche-les-Dames where it is claimed to have been found in 1934. These popular theories were supported by the fact that the King's death was illegally registered in Brussels and not in Marche-les-Dames [19], by the fact that the investigating magistrates have never seen the body in Marche-les-Dames - since it is claimed to be removed immediately after the discovery - or later in the Royal Palace, and by strong contradicting testimonies of the witnesses who found the body [Official juridical case files, Archive of the Royal Palace, Brussels, Belgium]. Also a translocation of the body to the place seven to nine hours after his death is unlikely to be in agreement with the huge amount of blood on several places uphill on the exact finding place, even on the climbing rock itself (Figure 2) and a large stone found close to the body, according to the juridical documents. A 'mise-en-scène' of the accident is thus highly implausible.

If at the time a commercial business in false blood samples was started, it is now clear that the present sample was none of those false relics. As mentioned by the newspapers – and as shown by the genetic identification of this sample – people have indeed collected such relics so that when a perimeter had been set by the gendarmerie, many traces would already have

disappeared for juridical investigation. This might be the reason that the first description of the scene of the accident mentioned the presence of large amounts of blood, while later descriptions do not [Official juridical case files, Archive of the Royal Palace, Brussels, Belgium]. In addition, since also the body had already been moved before the arrival of the police, the investigation by the authorities was already been seriously limited from the start, nourishing conspiracy theories.

Although the identification of the blood sample – forensic research that nowadays would be done in any case – is in line with the official result of the juridical investigation, a crime or suicide on the location cannot be excluded with this identification. Moreover, followers of conspiracy theories may implement the results of this study into new accessible stories to understand the ambiguous situation of the King's death, which is a distinctive element of conspiracy theories [56, 57].

ii) Ethical concerns in this biohistorical research

Already for a long time, debate exists on the privacy of a deceased individual [9, 29, 58]. However, even if the person is dead for a long time, the privacy of currently living descendants can be impacted when the genome of the deceased is analysed and the results are published. Here, this effect is strengthened, for the present and previous King of Belgium and the heirs are amongst those descendants, for whom inheritance is the basics of their royal function. Nowadays, much attention is paid to privacy and anonymity of genomic data [59, 60], but in identification studies it is by definition impossible to guarantee complete anonymity. Existing guidelines for dealing with privacy issues in similar biohistorical cases consider the balance between privacy and the scientific relevance of the research [10]. In this particular case where the historical and forensic value is undeniable, these guidelines are hitherto insufficient.

We opted in this research for a genetic genealogical approach to render objective evidence to the authentication of the blood sample. A complete genomic analysis on this historical DNA was technically feasible and could give information on the appearance of the person by e.g. facial reconstruction [61] and determining eye and hair colour [62, 63]. However, this allows only for a subjective interpretation or indication in an authentication analysis [3, 64]. Moreover, analysing functional DNA regions also provides information on physical traits, heritable illnesses or possible infertility. This is out of scope for our research objective and interferes

with questions which arise from mere curiosity [10]. Therefore, to protect the privacy of the person himself and his current relatives, we analysed only genetic regions that are relevant for identification, i.e. the mitochondrial DNA and (family-specific) Y chromosomal variation.

Despite a positive advice from the interdisciplinary review committee (KU Leuven – University of Leuven), we decided to withhold the Y chromosomal and complete mitochondrial genome data from publication to fully protect the privacy of living relatives. After all, the Y chromosomal data can be used to perform kinship analysis and mitochondrial genome has a phenotypic relevance. Many previous identification studies gave the mtDNA sequences and/or Y chromosomal data of anonymous reference persons [3, 65] and as a consequence the published data can be used to identify similar samples without reference persons or the consent of living relatives. Also the present sample could be partly authenticated using published data [38]. To avoid such situations, we decided to publish the names of the reference persons but without giving their DNA sequences. Although the mitochondrial control regions of several anonymous maternal descendants of the great-grand mother of Albert I were already published by Weichhold *et al.* [38], we do not publish the complete mtDNA genome sequences as they include coding regions and provide a much more accurate phylogenetic assignment in comparison with only control regions. Additionally, the research procedure, analysis and calculations and the lack of differences in the mitochondrial genome and the set of analysed Y-STRs between the samples have been described in detail. Since publication of the haploid data of the blood stains has the potential to violate the privacy of living relatives, our data and statistical interpretation were reviewed independently by forensic experts in haploid markers with no conflict of interest in order to guarantee the objectivity and scientific accuracy of the identification analysis. An additional privacy precaution we used in earlier genetic genealogical studies [53, 66] that has also been taken into account here is a distance of seven meioses between the King and the reference persons. Using this rule, if an extra-pair paternity event occurred in the past[67], it is impossible to point to the generation in which this event (likely) occurred. As soon as this manuscript is peer-reviewed, we will dispose of the data, the DNA extracts and the intermediate reaction products to prevent potential future misuse. Much attention has been paid to use a sampling technique on the blood relic as little destructive as possible to enable future research on the same relic. The sample has been

donated to an acknowledged foundation that will guarantee its appropriate preservation and protection.

iii) Conclusions

In the current post-genomic era where the technical possibilities are almost boundless, a plenitude of genomic data is produced. While this certainly brings additional value to genetic identification of biohistorical and forensic samples, care should be taken to preserve the privacy not only of the DNA donor himself, but also of his living relatives and cultural/ethnical stakeholders [10, 11]. We opted for an approach to limit the amount of data generated in genetic identification research (see Supplementary Materials). Only genetic data necessary for a correct identification should be sequenced, rejecting the data that give out of scope phenotypic or hereditary information and limiting what is communicated. After all, the interests of the relatives who have not provided their informed consent are not to be neglected. Hence, one of the major challenges in forensic genetics for the next decennium is to find an answer to these rising ethical concerns [68]. This study contains some preliminary outlines for the approach to balance privacy concerns against scientific interest in biohistorical and forensic analysis.

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Conflict of interest

The authors declare no conflict of interest.

Author's contribution

- Idea and supervision: MHDL
- Analysis: MHDL, BB, TW, DD and RD
- Writing: MHDL, MB and PB

Figures

Figure 1 Portrait of Albert I, King of Belgium (a) and place where his body has been found in Marche-les-Dames on 18 February 1934 (b). The X on photo (b) represents the exact place where the dead body was found according to the official version. Source: Wikicommons (a) and collection first author (b).

a)

b)

Figure 2 Photos of the juridical case files on the death of King Albert I made on 18th February 1934. (a) 'Photo 1 in the case files; this is the single photo of the exact place where the dead body of King Albert I was found according to the official version. The numbers, letters and arrows on the photos are noted by the investigating magistrates; with A, position of the cap of the King; B, position of leather straps of his knapsack; C, position of his knapsack; and D position of his carabiner; and both arrows indicate the position of a stone and leafs with blood traces; (b) 'Photo 8' in the case files with the rock whereon Albert I was exercising and had his climbing accident; with H, an overhanging rock with the presence of a tuft of hair as well as cerebral material particles embedded in small crevices. Source: Archive of the Royal Palace, Brussels, Belgium.

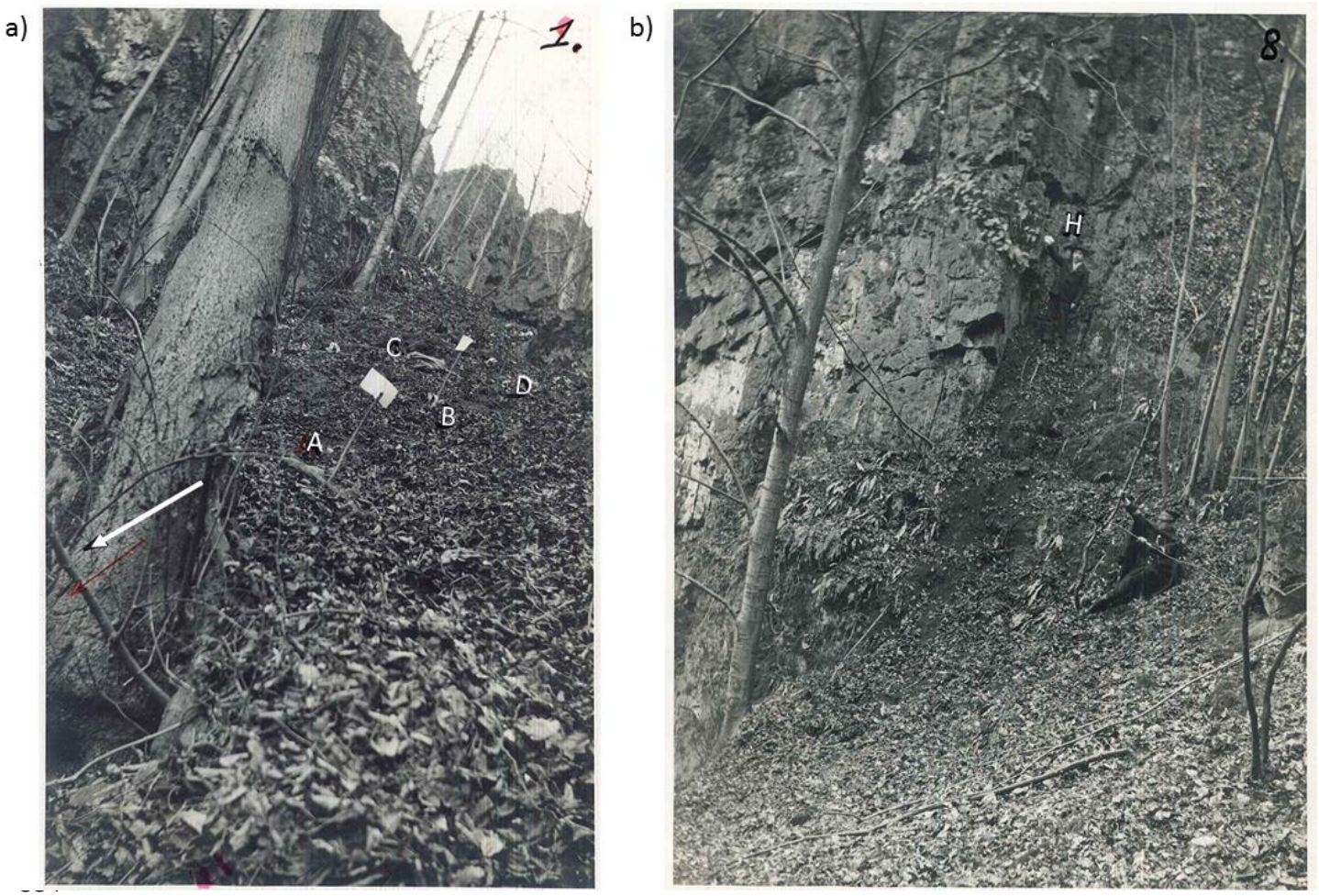


Figure 3 The sheet containing the blood-stained leaves. French handwriting on the front side reading “Leaves stained by the precious blood of HM Albert I. Marche-les-Dames 17 February 1934.”

Figure 4: Authentication certificate that was present between the two sheets and reading in French: “We undersigned authenticate the attached souvenir. Namur, 17 February 1934. M. de Thier born Facq, R. Goossens, d’…(*illegible*).”

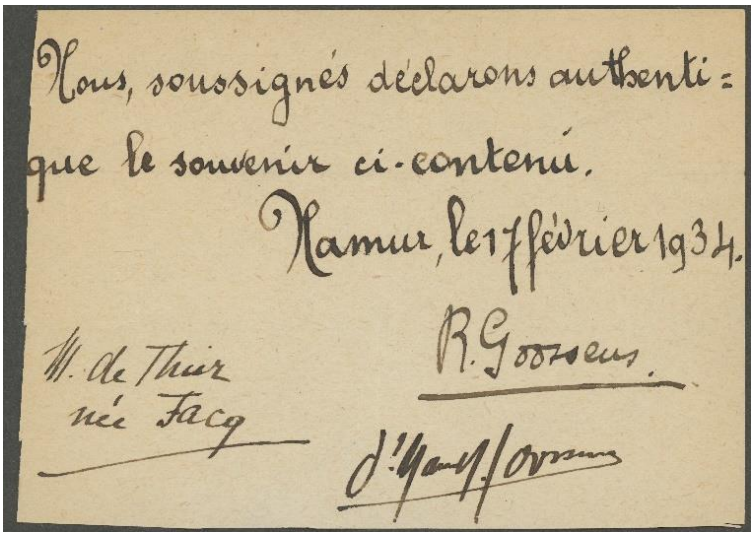
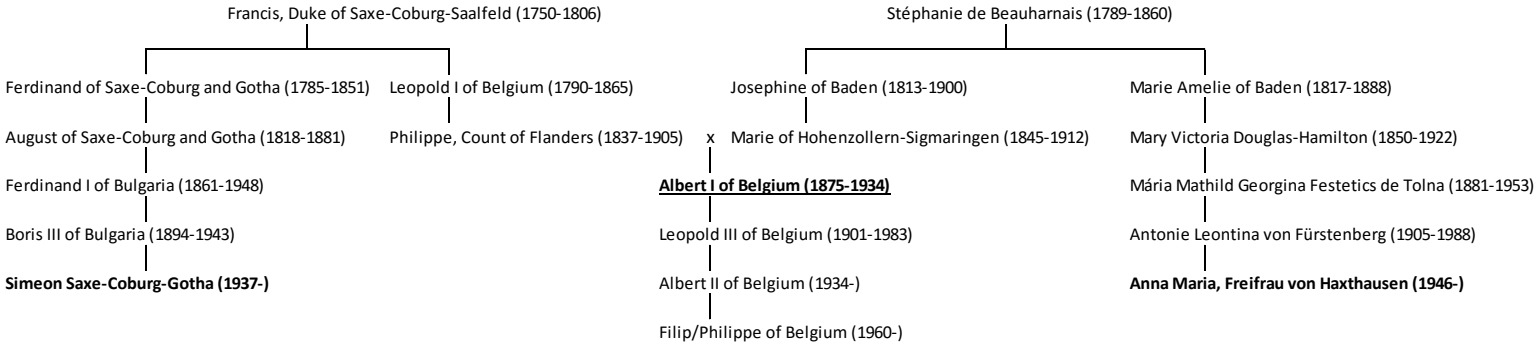


Figure 5 Genealogical relatedness between King Albert I of Belgium and his paternal and maternal relative (given in bold) as well with the current King of Belgium.



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